U.S. Serial No. 09/667,569 Attorney Docket: BGI-141CP

Group Art Unit: 1652 Examiner: D. Steadman

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1. (Original) A method of producing a panto-compound comprising culturing a microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme under conditions such that the panto-compound is produced.

Claim 2. (Original) The method of claim 1, wherein the microorganism overexpresses at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.

Claims 3-6. (Canceled)

Claim 7. (Original) A method of producing a panto-compound comprising culturing a ketopantoate reductase-overexpressing (KPAR-O) microorganism under conditions such that the panto-compound is produced.

Claims 8-11. (Canceled)

Claim 12. (Previously Presented) The method of claim 7, wherein the KPAR-O microorganism further overexpresses at least one pantothenate biosynthetic enzyme in addition to overexpressing ketopantoate reductase.

Claim 13. (Canceled)

Claim 14. (Withdrawn) A method of producing pantothenate in a manner independent of precursor feed comprising culturing an aspartate- α -decarboxylase-overexpressing (A α D-O) microorganism having a deregulated isoleucine-valine (*ilv*) pathway under conditions such that pantothenate is produced.

U.S. Senal No.: 09/667,569 Attorney Docker: BGI-I41CP

Group Art Unit: 1652 Exammer. D. Steadman

Claim 15. (Withdrawn) A method of producing at least 2 g/L pantothenate in a manner independent of aspartate or β -alanine feed comprising culturing an aspartate- α -decarboxylase-overexpressing (A α D-O) microorganism under conditions such that pantothenate is produced.

Claim 16. (Withdrawn) A method of producing at least 2 g/L pantothenate in a manner independent of valine or α -ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (ilv) biosynthetic pathway under conditions such that pantothenate is produced.

Claim 17. (Withdrawn) A method of producing at least 30 g/L pantothenate in a manner independent of aspartate or β -alanine feed comprising culturing an aspartate- α -decarboxylase-overexpressing (A α D-O) microorganism under conditions such that pantothenate is produced.

Claim 18. (Withdrawn) A method of producing at least 30 g/L pantothenate in a manner independent of value or ex-ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (ilv) biosynthetic pathway under conditions such that pantothenate is produced.



Claim 19. (Currently Amended) A β -alanme independent high yield production method for producing pantothenate comprising culturing a manipulated microorganism under conditions such that pantothenate is produced at a significantly high yield, as compared to an un-manipulated or wild-type microorganism.

Claim 20. (Withdrawn) The method of claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid synthetase or is transformed with a vector comprising an *thvBN* nucleic acid sequence or an *alsS* sequence.

Claim 21. (Withdrawn) The method of claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase or is transformed with a vector comprising an *ilvC* nucleic acid sequence.

Group Art Unit: 1652 Examiner: D. Steadman

U.S. Serial No.: 09/667,569 Attorney Docket: BGI-141CP

Claim 22. (Withdrawn) The method of claim 14 or 19, wherein the microorganism overexpresses dihydroxyacid dehydratase or is transformed with a vector comprising an *ilvD* nucleic acid sequence.

Claim 23. (Withdrawn) The method of claim 19, wherein the microorganism overexpresses aspartate- α -decarboxylase or is transformed with a vector comprising a panD nucleic acid sequence.

Claim 24. (Previously Presented) The method of claim 14 or 19, wherein the microorganism further has a deregulated pantothenate biosynthetic pathway.

Claim 25. (Withdrawn) The method of claim 14 or 19, wherein the microorganism further has at least one mutant gene selected from the group consisting of a mutant avid gene, a mutant ilvE gene, a mutant ansB gene and a mutant alsD gene.

Claim 26. (Original) The method of claim 24, wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate-or-decarboxylase.

Claim 27. (Previously Presented) The method of claim 24, wherein the microorganism is transformed with a vector comprising a panBCD nucleic acid sequence or a vector comprising a panE1 nucleic acid sequence.

Claim 28. (Previously Presented) The method of claim 14 or 19, wherein pantothenate is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

Claim 29. (Withdrawn) The method of claim 20, wherein the microorganism overexpresses acetohydroxyacid synthetase derived from *Bacillus* or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* nucleic acid sequence derived from *Bacillus*.

U.S. Serial No.: 09/667,569 Апотпеу Docker: BGI-141CP

Group Art Unit: 1652 Examiner D. Steadman

T-835

Claim 30. (Withdrawn) The method of claim 21, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase derived from *Bacillus* or is transformed with a vector comprising an *ilvC* nucleic acid sequence derived from *Bacillus*.

Claim 31. (Withdrawn) The method of claim 22, wherein the microorganism overexpresses dihydroxyacid dehydratase derived from *Bacillus* or is transformed with a vector comprising av *ilvD* nucleic acid sequence derived from *Bacillus*.

Claim 32. (Withdrawn) The method of claim 23, wherein the microorganism overexpresses aspartate-\alpha-decarboxylase derived from Bacillus or is transformed with a vector comprising a panD nucleic acid sequence derived from Bacillus.

Claim 33. (Previously Presented) The method of claim 24 or 26, wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate-\alpha-decarboxylase derived from Bacillus.

Claim 34. (Original) The method of claim 27, wherein the vector comprises a panBCD nucleic acid sequence or a panE1 nucleic acid sequence derived from Bacillus.

Claim 35. (Canceled)

Claim 36. (Withdrawn) A method of producing β -alanine comprising culturing an aspartate- α -decarboxylase-overexpressing (A α D-O) microorganism under conditions such that β -alanine is produced.

Claim 37. (Withdrawn) The method of claim 36, wherein the $A\alpha D$ -O microorganism has a mutation in a nucleic acid sequence encoding a pantothenate biosynthetic enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase and pantothenate synthetase.

Claim 38. (Canceled)

U.S. Serial No.: 09/667,569 Aποπικy Docket: BGl-141CP

Aug-07-03 17:11

Group Art Unit 1652 Examiner: D. Steadman

Claim 39. (Withdrawn) A method for enhancing production of a panto-compound comprising culturing a mutant microorganism having a mutant coaX gene under conditions such that the panto-compound production is enhanced.

Claim 40. (Withdrawn) The method of claim 39, wherein said recombinant microorganism has a mutant coaA gene.

Claim 41. (Withdrawn) A method of producing a panto-compound comprising <u>culturing</u> a pantothenate kinase mutant microorganism under conditions such that the panto-compound is produced at a significantly high yield.

Claim 42. (Withdrawn) The method of claim 41, wherein said mutant microorganism has a mutant coast gene.

Claim 43. (Withdrawn) The method of claim 41, wherein said mutant microorganism has a mutant coaX gene.

Claim 44. (Withdrawn) The method of claim 41, where said mutant microorganism has a mutant coast and coast gene.

Claim 45. (Canceled)

Claim 46. (Withdrawn) The method of claim 39 or 41, wherein said panto-compound is pantothenate.

Claim 47. (Withdrawn) The method of claim 39 or 41, wherein said panto-compound is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

Claim 48. (Withdrawn) The method of claim 39 or 41, wherein said recombinant microorganism further has a deregulated pantothenate biosynthetic pathway or further has a deregulated isoleucine-valine (ilv) biosynthetic pathway.

Claim 49. (Previously Presented) The method of claim 39 or 41, wherein said recombinant microorganism further overexpresses panD and panE.

U.S. Serial No.: 09/667,569 Attorney Docket: BGI-141CP

Aug-07-03 17:11

Group Art Unit: 1652 Examiner: D. Steadman

Claim 50. (Withdrawn) The method of claim 39 or 41, wherein said recombinant microorganism further has at least one mutant gene selected from the group consisting of a mutant aviA gene, a mutant ilvE gene, a mutant ansB gene and a mutant alsD gene.

Claim 51. (Previously Presented) A method for enhancing production of a panto-compound comprising culturing a microorganism that has a deregulated pantothenate biosynthetic pathway and that also has a mutation that results in reduced pantothenate kinase activity under conditions such that the panto-compound production is enhanced.

Claims 52-110. (Canceled)

Claim 111. (New) A method for producing a panto-compound comprising:

- (a) transforming a microorganism with a vector to produce a recombinant microorganism, wherein said vector comprises a panE gene with 95% or greater amino acid identity to SEQ ID No:30, operably linked to a constitutively active promoter set forth in SEQ ID NO:40, and further operably linked to a regulatory sequence comprising an artificial ribosome binding site (RBS), set forth in SEQ ID No:50;
- (b) growing said recombinant microorganism in an appropriate culture medium and under conditions suitable for the production of said panto-compound; and
 - (c) recovering said panto-compound from said culture medium.

Claim 112. (New) A method for producing a panto-compound comprising:

- (a) transforming a microorganism with a vector to produce a recombinant microorganism, wherein said vector comprises a panE gene with 95% or greater amino acid identity to SEQ ID No:30, operably linked to a constitutively active promoter set forth in SEQ ID NO:40, and further operably linked to a regulatory sequence comprising an artificial ribosome binding site (RBS), set forth in SEQ ID No:50;
- (b) further transforming said recombinant microorganism with a vector comprising a panBCD operon with 95% or greater amino acid identity to SEQ ID No: 24, 26, and 28, operably linked to a constitutively active promoter set forth in SEQ ID



Aug-07-03 17:11

U.S. Serial No.: 09/667,569 Aπoraey Docket: BGI-141CP

Group Art Unit: 1652 Examiner: D. Steadman

NO:40, and further operably linked to a regulatory sequence comprising an artificial ribosome binding site (RBS), set forth in SEQ ID No:50;

- (c) growing said recombinant microorganism in an appropriate culture medium and under conditions suitable for the production of said panto-compound; and
 - (d) recovering said panto-compound from said culture medium.

Claim 113. (New) The method of claim 112, wherein said recombinant microorganism is further transformed with a plasmid set forth in SEQ ID No: 78. (to overexpress panD)

Claim 114. (New) The method of claim 113, wherein said recombinant microorganism is further transformed with a plasmid set forth in SEQ ID No: 82. (to deregulate ilvBNC)

Claim 115. (New) The method of claim 114, wherein said recombinant microorganism is modified to deregulate the *Bacillus subpilis ilvD* gene.

Claim 116. (New) The method of any one of claims 111-115, wherein said appropriate culture medium contains valine.